intended appears, however, to have remained intact.

I would like now to introduce the first speaker of this session, Dr. David Platt Rall. Dr. Rall is the Director of the National Institute of Environmental Health Sciences. He has been in that position since 1971. Since 1971 he has also been Assistant Surgeon General of the U. S. Public Health Service. Dr. Rall holds both an M.D. and a Ph.D. in Pharmacology from Northwestern University. Currently he is U. S. Coordinator, Environmental Health Program, U. S.-USSR Health Exchange Agreement and a member of the Editorial Board, Pharmacological Reviews. He is also a member of the Graduate Council of the George Washington University. Dr. Rall has authored over 100 published papers relating to comparative pharmacology, cancer chemotherapy, blood-brain barrier, blood CSF barrier, pesticide toxicology, and drug research and regulation.

Problems of Low Doses of Carcinogens

David P. Rall, M.D., Ph.D.

National Institute of Environmental Health Sciences, P.O. Box 12233, Research Triangle Park, No. Car. 27709

My assigned topic today is the question of how to assess for carcinogenic potential those chemicals that we find in our environment. It is, I suspect, unnecessary to dwell on the problem of cancer as a serious public health threat. There have been estimates suggesting that as much as 80% of the cancer in man in the United States is related to environmental chemical factors. It becomes really of enormous importance to eliminate as much as possible, as much as feasible, carcinogenic compounds from the environment. This area of discussion has in the past, and I am sure will in the future, often generate rather more heat than light, particularly with respect to the role of animal testing in environmental carcinogenesis. The classic statement is that the proper study of mankind is man. I think there is an undercurrent of feeling amongst some people that perhaps the use of animals studied appropriately or inappropriately in carcinogenicity testing is not as necessary as it is claimed to be. It seems to me that we must in fact use animal tests, today at least, as the basis for prediction of carcinogenic activity. Surveillance of the human population or selected subsets of the population for incidents of tumors is very, very important; but this is a last resort. If, in fact, an agent does enter the environment that does cause cancer in man, by the time it would be detectable in any sort of reasonable disease surveillance system, we would already have a massive epidemic of environmentally caused carcinogenesis. It is too late a point in time to have identified the carcinogen. Secondly, the manpower resources in the United States in terms of chronic epidemiology are so woefully weak in numbers—not in quality, but in numbers—that it would simply be unrealistic to view, any time in the near future, epidemiology taking on any more than it is doing right now. This is a matter of fact, an urgent national problem, that I hope can be addressed as soon as possible. We simply do not have enough capability in chronic epide-

miology, and we must begin to get more. Finally, the view is that if cancer is proven in man, and everybody agrees that the compounds are carcinogenic in man, this would tend to end controversy. I think history would prove that this is not true. Some of you may be familiar with the University Group Diabetes Program, which seemed to an innocent non-epidemiologist like myself a reasonably designed and executed prospective study with a rather straightforward outcome. It is inconceivable to believe that anything involved in carcinogenesis would generate less controversy than that study evolved. Therefore we are stuck with animal studies.

I would like to spend my time describing the problems of using animal studies to extrapolate to man. I shall concentrate more on problems of comparative pharmacology, physiology, and toxicology and leave the statistics to Marvin Schneiderman. (On the other hand, he isn't going to talk about statistics either.)

Fig. 1 presents a way of looking at this

I. Median mouse vs. median man

II. Genetic and environmental heterogeneity in man

Fig. 1. Assessment of environmental chemicals for carcinogenesis.

which I shall try to develop—that is, trying to take results from a well-conducted animal study and applying them to man. There is, first, the systematic differences between the species that you are looking at in the laboratory, the mouse, and the species that you are trying to extrapolate to, in this instance, man. I would like to divide this up into first a "median mouse" to "median man" consideration. That is, in a very homogeneous population under strict environmental control, what are the differences in response between a very small mammal with its own peculiar set of metabolic processes and a relatively large mammal, a man with his own peculiar set of physiological, biochemical, and pharmacological processes? This is the first step. The second step then is to look at the final organism we are trying to protect; that is, one individual person in a very large population, a very diverse population within the United States. Here we must get into the genetic and environmental heterogeneity in man.

To make discussion smoother I would like to present this in a somewhat different organizational rubric where I would like first to consider some differences and sensitivity in laboratory animals with respect to pharmacologic receptor differences, temporal, and size differences; then discuss some problems of population difference; and then very briefly some problems of environmental differences.

Fig. 2 shows some problems of

I. Sensitivity of laboratory animals as compared to man
   A. Pharmacological differences
   B. Receptor differences
   C. Temporal differences
   D. Size differences

II. Population differences
   A. Size
   B. Heterogeneity
   C. Selected nature of test population

III. Environmental differences
   A. Nutritional
   B. Physical
   C. Chemical

Fig. 2. Assessment of environmental chemicals for carcinogenesis—differences between test animals and man.

pharmacological differences between one species and another. We must recognize that before a compound acts at its final site of action, whether this be a compound interacting with DNA in a bone marrow cell to initiate a leukemia or what, there are a variety of steps that compound must pass through before it reaches this final site of action in its final chemical form. Each of these steps from absorption and distribution to metabolism and excretion and finally its arrival past some variety of cell barriers and its ultimate interaction with that final receptor enzyme or chemical can vary from one animal species to another. Some vary in a predictable way. Briefly, it is rather well known that absorptive mechanisms are not terribly different between various species. One interesting problem is the different hydro-

gen ion concentrations in the stomach of some of the herbivorous and carnivorous animals. I shall come back to this problem of distribution later because this seems to be more a function of size than a species difference. Now metabolism—the xenobiotic metabolism of foreign compounds—differs greatly from species to species. Some recent work is beginning to suggest some general principles in the differences which may be of importance. It is quite clear that herbivores in general have a much more active xenobiotic metabolism system than carnivores. This was perhaps first brought to our attention when the veterinarians in the zoo tried to anesthetize a tiger with pentobarbitol (which works very well with small mammals). The tiger fell asleep promptly but never woke. Since this was a prized animal, comparative pharmacology became quite important. The metabolic patterns are increasingly important because we are beginning to realize more and more clearly that very often the compound that was administered is not the ultimate carcinogen, and it takes metabolic processes within the body to create the active compound. There are some differences in excretory rates between species but these do not seem to be of major importance. The various cellular and intracellular barriers seem to be surprisingly constant throughout the vertebrate kingdom. With regard to receptor differences and the ultimate mode of action, it seems that this is surprisingly constant in the vertebrate kingdom. A molecule of DNA from a mouse, a rat, or from a man is not very different, and the interactions of that molecule with chemicals which come ultimately from the environment are surprisingly similar. However, there are temporal differences which I think have not been considered in the past. It takes time to develop a tumor, and at least some of that time is related to the actual cell division process. The renewal rate of the bone marrow or of the gastro-intestinal tract of the mouse can be compared with the rate in man. The cell division rate is significantly faster in small animals. The cell cycle times are about half, the cell turnover periods are about double in man. Mice and rat cells turn over faster. The latent period for the development of tumors is faster in mice and rats. One example of this is shown in Fig. 3, the latent period for the development of thyroid tumors after radiation iodine administration in the rat, the dog, and man. Rats developed the tumors in the order of 1–1½ years, dog with a spread from 4–10 years, and man took close to 12 years to develop the tumor. There is apparently a systematic difference in the latent period related to body size.

It is important also to realize that the life span of man is about 35 times that of mouse or rat. How can we put this all together? The cell division time is twice as fast in the smaller animal, so there is in a sense twice as great a chance for some untoward event to happen. The more rapid cell division rate in part must account for the shorter latent period in the very much smaller animals. However, the life span of man is so much longer indeed that there is much longer lifetime opportunity to develop a tumor. I would suggest that what I have run through is a very simplistic view of these temporal differences. But I think in the future we should spend more time considering them as we consider the implications of

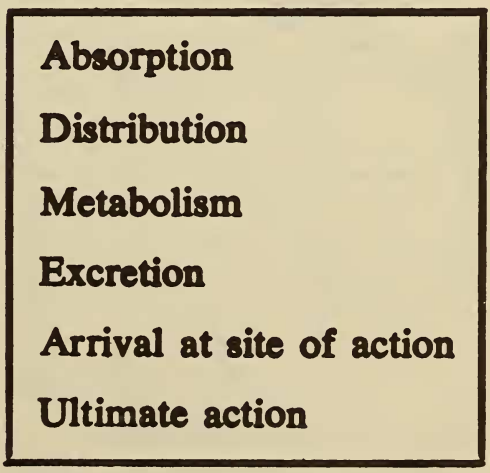

Fig. 3. Steps a drug must pass before it can act.

lifetime studies in small animals for lifetime exposure in large animals. I think as we learn more about the actual mechanism of carcinogenesis in experimental studies, this view of the temporal differences between very small and very large animals might be very useful indeed.

Now let us move into problems of size differences. The size determines in many ways the rate of distribution of foreign compounds throughout the body. To take a very simple example, the blood volume of a mouse is about 1 ml. The cardiac output of that mouse is about 1 ml./m. The mouse turns over its blood volume in about 1 minute. In man the cardiac output is only about 1/20 of the blood volume in man. The mouse moves things around about 20 times faster than man. Thus, the exposure of a tissue to a compound in a small animal occurs more rapidly. But excretion also would be much more rapid, and on a weight basis small animals excrete compounds more rapidly. Therefore, it is reasonable to expect that small animals would be able to tolerate larger doses of compounds. Fig. 4 shows the toxic doses of a number of anti-cancer drugs, to compare, not on a weight basis, but on either a surface area or a weight to two thirds power basis. There is reasonably good agreement between the human toxic dose and the BFD_1 mouse toxic dose. In essence man is about 12 times more resistant than the mouse. There is another aspect to this slower rate of distribution and metabolism in the large animals that I think is important, and that is in terms of long-term studies. Fig. 5 presents a mixture of data from the U. S. Market Basket Survey, from the Pesticide Survey on the human levels of DDT, and from an IARC (Lyon) report on the fat and tissue levels of DDT in a carcinogenesis experiment in mice. The intake of the mouse was 2 ppm DDT in the diet. This was about 6–8 μg/day, or about 3 mg/year. Man, according to the Market Basket Survey, 3 or 4 years ago ingested about 30 μg/day of DDT or a total of about 10.8 mg/year. The total intake in the mouse over 1 to 2 years of the experiment was about 5 mg, or a total of 100 mg/kilo. The average DDT concentration in the fat of the mice at sacrifice at the end of the experiment was 5 to 6 ppm. Man in his 20 to 30 years' exposure to DDT had a quarter of a gram or about 4 mg/kgm total exposure; but this steady state fat concentration on the average was about the same or about 5 to 6 ppm in the fat. We need to know more about the final concentration of the compound in the experimental animal and the exposed human population.

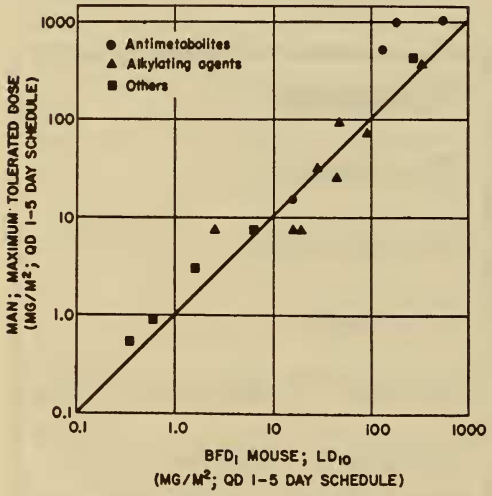

Fig. 4. Toxic doses of a number of anti-cancer drugs.

Average USA Man and Average Mouse on 2 ppm in Diet

Intake	Mouse	10 ug/day	3.6 mg/year
	Man	30 ug/day	10.8 mg/year
Total Intake	Mouse	1-2 years	5 ± mg or 200 mg/kg
	Man	20-30 years	250 ± mg or 4 mg/kg

DDT Concentrations in fat = 5-6 ppm in man and mouse.

Fig. 5. DDT intake in mice and man.

There is one other aspect to the size difference which I would like to touch on very briefly. The large animal has a very much larger number of susceptible cells in his body that may interact with the potential carcinogenic agent. For instance, there are from 160 to 2000 times more susceptible cells in one man than in

one mouse. Thus, one man is equivalent to at least a 160-mouse experiment. If there is a relationship between the initiation of a carcinogenic event and the number of susceptible cells, and this to me is logical, then one man is possibly more sensitive than one mouse.

Let us now move on to population differences. The first problem, one that has been extensively discussed, concerns the problem of extrapolating toxicity or carcinogenicity results from a few hundred laboratory animals to a few hundred million people. Another major problem is the heterogeneity of the human population. I believe Fig. 6 illustrates this very well. What is shown is the steady state plasma level of a tricyclic antidepressant given to a number of patients at the standard clinical dose after allowing a steady state to develop. In this random group of patients the plasma concentrations at steady state varied from about 10 μg/l to 300 μg/l in plasma, an enormous variability. So it is pertinent to ask, if one is trying to extrapolate data from a laboratory experiment to man, does the laboratory experiment reflect those patients on the far left corner, the middle, or the right corner? There can be very great differences. I have shown this only for the metabolism of this one drug. The body rids itself of foreign organic compounds largely by metabolic rather than purely excretory mechanisms. This is largely a difference of xenobiotic metabolic pathways, yet every aspect of the handling of a compound by the body is potentially involved in such human heterogeneity.

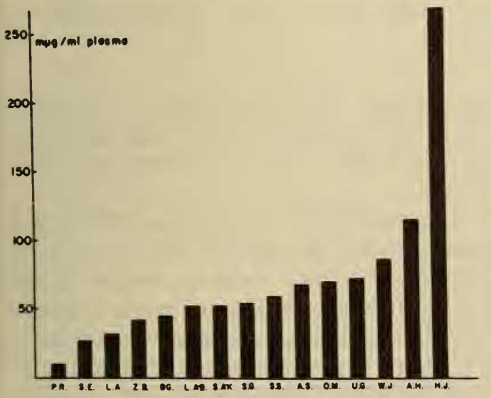

Fig. 6. Steady state levels of NT during daily oral dosage of 3 × 25 mg.

It is also necessary to consider the very selective nature of the test population. Laboratory scientists go to all ends to select vigorous, well fed, healthy animals to extrapolate to a population which contains sub-populations that have all varieties of illness, weakness, and disease. Thus, population differences related to size, to genetic heterogeneity, and to the very selected nature of the test population are important.

Finally, there are environmental differences which I shall touch on briefly. I think many of these are obvious. Nutritional differences clearly can alter response to carcinogenic agents. This is well documented. The laboratory animal is on a diet that is well supplemented with vitamins, minerals, adequate proteins, and so forth, while many segments of the American population have diets of varying quality. The possibility of significant differences is apparent there. The physical environment—heat, light, ionizing radiation, etc.—can affect responses. Again we know the very great difference between a well controlled animal room and the human situation. Perhaps the major problem is the chemical environment. The proper laboratory scientist makes every effort to be sure there are no mycotoxins in the feed for his animals and that there are no nitrosamines in the feed for his animals; the next morning he sits down and has bacon for breakfast. With the various potentially toxic compounds in air and water and food and with concurrent drug administration there exists a great opportunity for synergistic toxicity. This is a problem that is only beginning to be approached in the environmental field. In the field of therapeutic drugs, the joint toxicity of two drugs has been demonstrated many times.

These differences, nutritional, physical, chemical, and environmental, all must be considered in any attempt to use laboratory animal toxicity or carcino-

genicity data to extrapolate to man. The net result of all of these differences suggests to me at least that the laboratory animal is not a sensitive indicator of carcinogenicity in tests with environmental chemicals. If results from laboratory animal tests are to be used to set up guidelines to protect very large human populations it is prudent to be extremely conservative in trying to apply this extrapolation.

Another way of looking at this is shown in Fig. 7. Some of you may have read an article in *Science* about seven years ago about some behavioral scientists who had been studying LSD in the cat and wished to see what happened in the elephant. They gave the mg/k dose of LSD which provided whatever behavioral response they wanted in the cat to an elephant borrowed from one of the local zoos. The result in a relatively few minutes was a very, very large elephant convulsing, defecating, and finally dying. What I would like to suggest is that we must not forget this principle of comparative pharmacology and toxicology as we try to extrapolate data from laboratory animals to man, or we may be associated with a very large convulsing and defecating elephant.

Fig. 7. "I just got tired of rats and mice, rats and mice."

Safe Dose? Problem of the Statistician in the World of Trans-Science

Marvin A. Schneiderman, Ph.D.

National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20014

When the statistician works on an issue in the public arena he often finds that the data he collects, and the manner in which he analyzes the data are conditioned by things outside his own professional competence. This paper gives some examples that attempt to discuss what the statistician might do that despite these pressures he might provide, if not an unbiassed picture, at least a fuller picture. Because I am from the National Cancer Institute, I am mainly concerned with the problems of what causes cancer, how we determine that a material is a carcinogen, and the statistician's role in establishing "safe" doses, if there are such things.

The statistician is constrained by the biological models of his laboratory colleagues. If the research worker with whom you are working is of the opinion that there is threshold in carcinogenesis, i.e. there are some doses that are sufficiently low so that they will not produce any cancer whatever, then it is extremely likely that he will design experiments